

SPECIATION IN UVULARIA

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With plates 82 and 83 and five text figures

I. A CYTOLOGICAL SURVEY OF UVULARIA

THE PURPOSE of this section of the investigation was to determine by cytological examination the evolutionary importance of certain phenomena already known to occur in the genus *Uvularia*. Belling (1925) had shown that in *U. grandiflora* non-disjunction, lack of pairing, fragmentation of chromosomes, and the duplication of single chromosomes or the entire chromosome complement may take place under experimental conditions. Our problem was to determine whether in the genus *Uvularia* these phenomena are of importance in the differentiation of species. If chromosome duplication has been one of the causes of specific differentiation, these two species of *Uvularia* should differ in their chromosome number. If the entire chromosome set has been duplicated, one would expect to find polyploid strains within the species, such as have been reported for *Tradescantia* (Anderson and Diehl 1932), *Tripsacum* (Mangelsdorf and Reeves, 1931) and other genera. If chromosome fragmentation has been involved in species differentiation, one would expect to find single individuals or whole geographic races characterized by the possession of fragmented chromosomes.

The material is excellent for such a study. The species under consideration have well-marked specific differences and the chromosome complement of one species has been worked out in detail (Belling, 1925). It has seven (n) chromosomes, each of which can be identified by its morphological peculiarities. Therefore if gross chromosomal differences are responsible for specific differentiation in this genus, the point should be rather easily detected.

An effort was made to obtain plants for cytological examination from as many widely separated points as possible. *Uvularia grandiflora* was collected at five points and *U. perfoliata* at two. The data are summarized in Table I.

TABLE I.

SPECIES	LOCALITY	NO. OF PLANTS EXAMINED	CHROMOSOME NUMBER (n)
<i>U. grandiflora</i>	Herculaneum, Mo.	2	7
	Cliff Cave, Mo.	3	7
	Farmington, Ark.	3	7
	Schoolcraft, Mich.	1	7
	Hamilton, N. Y.	1	7 + f
<i>U. perfoliata</i>	Great Smoky Mts., N. C.	2	7
	New Canton, Virginia	3	7

As shown in Figure 1 there are no evident differences in chromosome size or configuration between the species, and there is only one case of an intra-specific difference, the plant examined at Hamilton, N. Y. For this observation we are indebted to Dr. C. L. Stebbins, who kindly made a cytological examination of local material. He found the characteristic chromosome complement for the genus, and in part of the microspores an additional fragment chromosome, much smaller than the others and similar to those reported by Belling (1925). Fragmentation, therefore, can and does take place in nature as well as in the laboratory, though there is no evidence that it has been of any importance in the differentiating of species within the genus *Uvularia*.

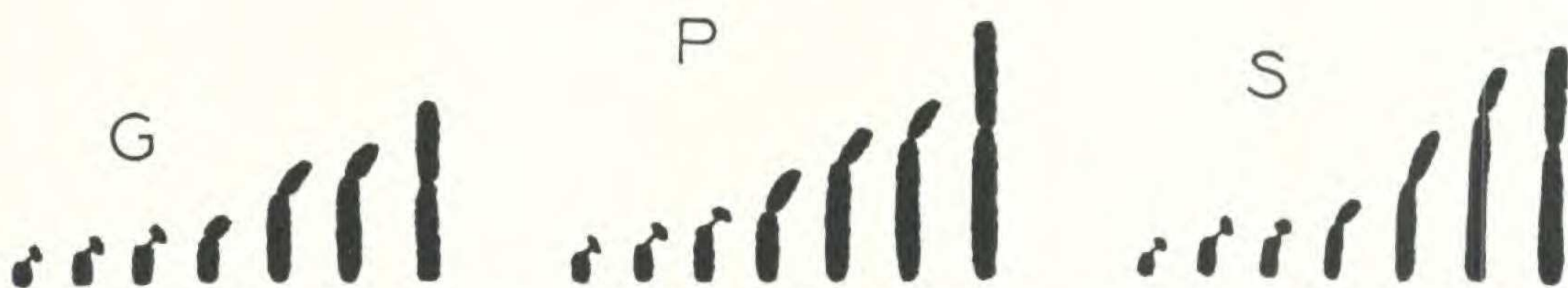


FIGURE 1. Chromosome complements of *Uvularia grandiflora* ('G'), *U. perfoliata* ('P'), and *Oakesia sessilifolia* ('S'), redrawn diagrammatically from the original camera lucida sketches.

The investigation was extended by examining the chromosome complement of the closely related *Oakesia sessilifolia* (L.) S. Wats. (*Uvularia sessilifolia* L.)

Plants were collected in the vicinity of Boston and the somatic chromosomes were studied in root tips. Fourteen chromosomes are present and it is possible to identify the seven types. Figure 1 (S) is a diagrammatic drawing of the seven, made to scale from camera lucida sketches.

CONCLUSION: There is no evidence that chromosome duplication or chromosome interchange are of phylogenetic importance in *Uvularia*.

Although these phenomena are known to have occurred under experimental conditions and may well occur in nature, they apparently have no direct evolutionary significance in this genus. So far as can be determined by cytological examination, specific differentiation in *Uvularia* has not involved gross differences in the number or relative size of the chromosome complement. This conclusion is strengthened by the fact that *Oakesia sessilifolia*, although usually classified in another genus, has a chromosome complement essentially identical with that of the two species of *Uvularia*. These results are of particular interest in view of the data presented in Part II, which show that *U. grandiflora* and *U. perfoliata* are well differentiated species. We do not mean to suggest that chromosome duplication and interchange are never of phylogenetic importance but merely call attention to the fact that there are groups of plants, like the genus *Uvularia* in which they are not species-forming forces.

II. A MORPHOLOGICAL SURVEY OF UVULARIA

The following morphological survey of *Uvularia grandiflora* Sm. and *U. perfoliata* L. is an attempt to present objectively, in a codified form the essential facts as to resemblances and differences within and between these two similar but distinct species. It is an attempt to reproduce in a concise manner, for non-taxonomists, the kind of data which are consciously and unconsciously used by taxonomists in the delimitation of species. Fundamental biological unit though it may be, the Linnean species is still definable only by example (definable that is in terms which carry meaning to workers in other scientific fields). Those biologists who in the herbarium and in the field have had the opportunity to study a number of such examples, have come to an appreciation of the importance of these fundamental units and to a general notion of their relative magnitude, as compared with individual differences on the one hand and generic differences on the other. There are, however, many non-taxonomists who though they do not have the time nor the training to participate in taxonomic work would like to gain a rough working knowledge of a Linnean species. If a species cannot as yet be defined in terms which are meaningful to workers in other fields of biology, one can at least present the range of variation within and between two closely related species in such a summarized form that the results may be digested in a comparatively short time.

The following pages present such a summary. It is not a taxonomic treatise on the two species. They were chosen for study not because their taxonomic position was in doubt, *but for precisely the opposite*

reason. They were taken as an example because there seemed to be a very general agreement, on the one hand that they were specifically distinct from one another, and on the other hand that they were closely related members of the same genus. As far as practical taxonomic work is concerned, the data reported below merely confirm the customary disposition of *Uvularia grandiflora* and *U. perfoliata* as two distinct species of one genus.

After a preliminary study of herbarium material 15 sheets of each species were selected from the collections at the Gray Herbarium and were subjected to intensive study. Several times as many would have been desirable but we were obliged to limit the sample in order to obtain well-developed material in which the characters chosen for study could be measured accurately. The following collections were studied:

Uvularia grandiflora

C a n a d a : Quebec, Lake Memphremagog, dry ground, *J. R. Churchill*, Aug. 17, 1914. V e r m o n t : Middleburg, *E. Brainerd*, May 18 - June 5, 1879; Shell House Mt., Ferrisburg, *E. and C. E. Faxon*, June 19, 1891; Hubbardston, *W. W. E.*, no. 2, July 17, 1898; Hyde Manor, Sudbury, *Geo. G. Kennedy*, May 23, 1908; Manchester, *M. A. Day*, no. 179, July 7, 1898; near Hyde Manor, Sudbury, *E. F. Williams*, May 23, 1908. N e w Y o r k : North Fort Ann, Washington Co., *S. H. Burnham*, May 30, 1920. I l l i n o i s : Starved Rock, La Salle Co., in rich woods, *J. M. Greenman et al.*, no. 26, June 1-7, 1909. W i s c o n s i n : St. Croix Falls, *C. F. Baker*, July 8, 1900; *J. M. Greenman*, no. 2165, June 5, 1907. M i s s o u r i : Taney Co., rich woods, along rocky bluffs, *E. J. Palmer*, no. 19237, Sept. 29, 1920; Creve Coeur Lake, *M. W. Lyon*, June 12, 1904; Clark Co., *B. F. Bush*, no. 2, Aug. 26, 1892; Cape Girardeau Co., rich woods along Miss. River, *E. J. Palmer*, no. 17998, June 21, 1920.

Uvularia perfoliata

M a s s a c h u s e t t s : Sudbury, *E. F. Williams*, no. 1, May 30, 1900; Sudbury, *E. F. Williams*, no. 1, May 30, 1900; Granville, Hampden Co., deep rich, deciduous wood, *F. C. Seymour*, no. 191, June 24, 1914; South Georgetown, open woods, *E. F. Williams*, Aug. 1, 1899. C o n n e c t i c u t : Southington, shade, *L. Andrews*, no. 358, June 3, 1898; Cromwell, *E. Wright*, June 7, 1878. N e w Y o r k : Tompkins Co., in woods, *A. Gershoy*, no. 7845, June 27, 1917; South Nyack, *L. M. S.*, June 2, 1888. P e n n s y l v a n i a : Berks Co., woods, eastern slope, *H. B. Meridith*, June 14, 1922; Delaware Co., woods,

A. MacElwee, no. 97567, June 16, 1899. New Jersey: Somerdale, Camden Co., rich wooded slope, *H. B. Meridith*, May 27, 1921. Virginia: Bedford Co., *A. H. Curtiss*, Aug. 26, 1871. District of Columbia: Washington, *E. S. Steele*, May 4, 1896. North Carolina: Waynesville, *E. E. Magee*, June 9, 1897; Asheville, *B. L. Robinson*, no. 75, Aug. 2, 1893.

A number of interesting characters could not be studied, either because they were poorly preserved or because they were present on only a few of the sheets. For these reasons no detailed observations are reported on roots, seeds, fruits, and scale leaves (cataphylls).

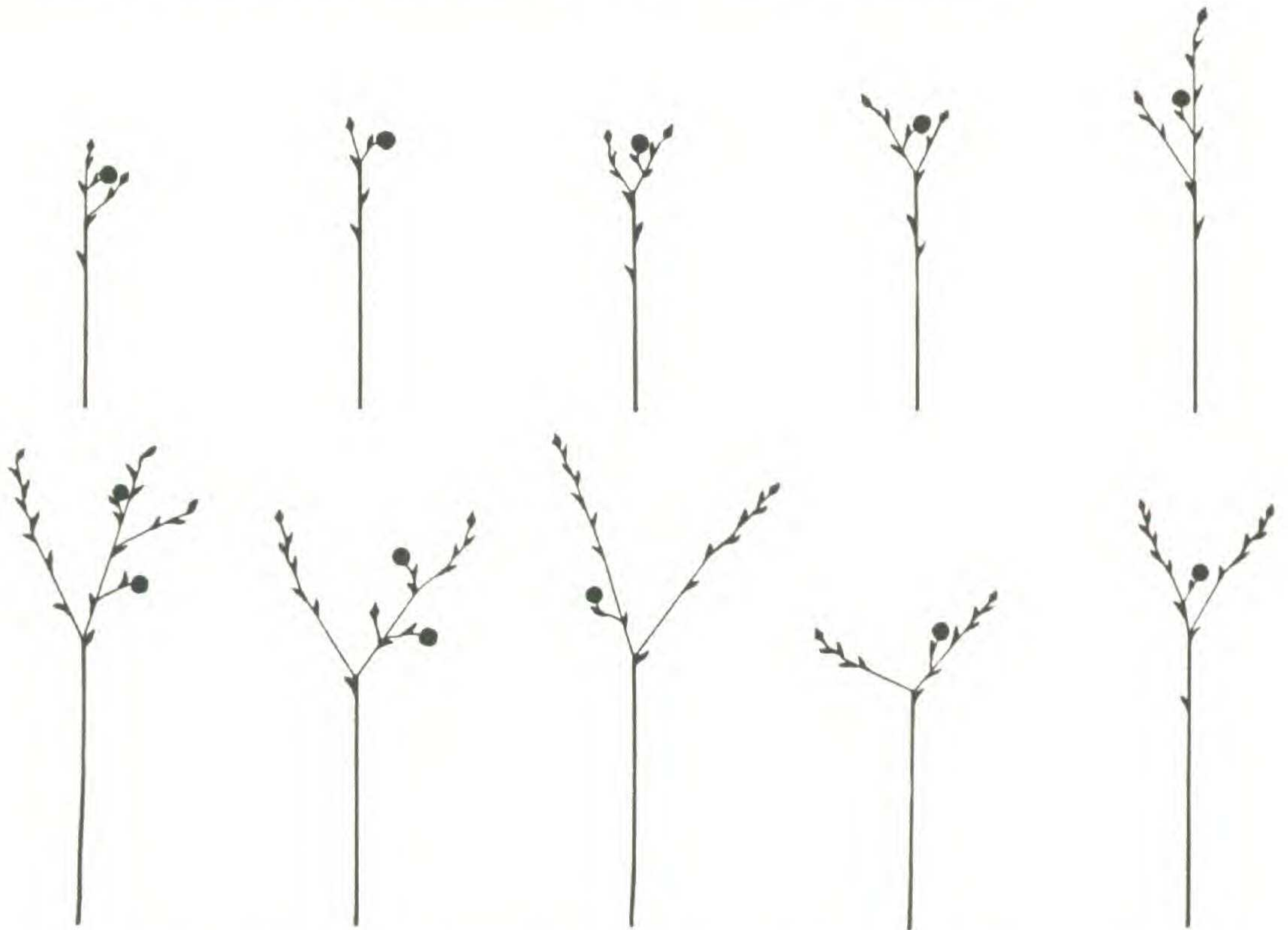


FIGURE 2. Diagrams, to scale, showing node number, etc. in 5 specimens of *U. perfoliata* (above) and 5 of *U. grandiflora* (below). Further explanation in the text.

STEM.

Five flowering specimens of each species are shown diagrammatically in Figure 2. A few poorly preserved details (such as the number of scale leaves at the base of the stem) have been ignored. The drawings are made to scale so far as internode lengths are concerned, and the angles of the branching have been approximately reproduced. The representations of the leaves and flowers are purely conventional. A

study of Figure 2 reveals the following differences between the two species.

- A. *U. grandiflora* is somewhat larger, on the whole.
- B. *U. grandiflora* has fewer nodes below the lowest branch.
- C. *U. grandiflora* has more nodes on the lowest (sterile) branch.
- D. *U. grandiflora* has a higher average number of flowers per plant.
- E. In *U. grandiflora* the upper end of the main axis is less perpendicular than in *U. perfoliata*.

TABLE II.

Number of nodes	0	1	2	3	4	5	6	7	8
Number of nodes with leaves below branch									
<i>U. perfoliata</i>			5	9	1				
<i>U. grandiflora</i>		7	8						
Number of nodes on sterile branch									
<i>U. perfoliata</i>		1	9	4	1				
<i>U. grandiflora</i>					1	4	7	1	2

Of these five differences, B and C are relatively unaffected by the age of the plant and can be readily ascertained from herbarium specimens. They were chosen accordingly for more extensive investigation and are recorded in Table II, for all of the specimens under consideration. It will be seen that when a number of specimens are considered that neither character is discontinuous. This is true of all five of the characters of the stem enumerated above. After a little study any single specimen could be correctly placed, by a combination of these five characters, no one of which would suffice, if taken separately.

LEAVES.

Tracings of leaf outlines from the same five specimens are presented in Figure 3. To make the comparison more exact a particular leaf was

chosen, namely, the leaf located at the axis of the main branch and the fertile shoot.

The following differences, or tendencies to differ, can be found between leaves of the two species.

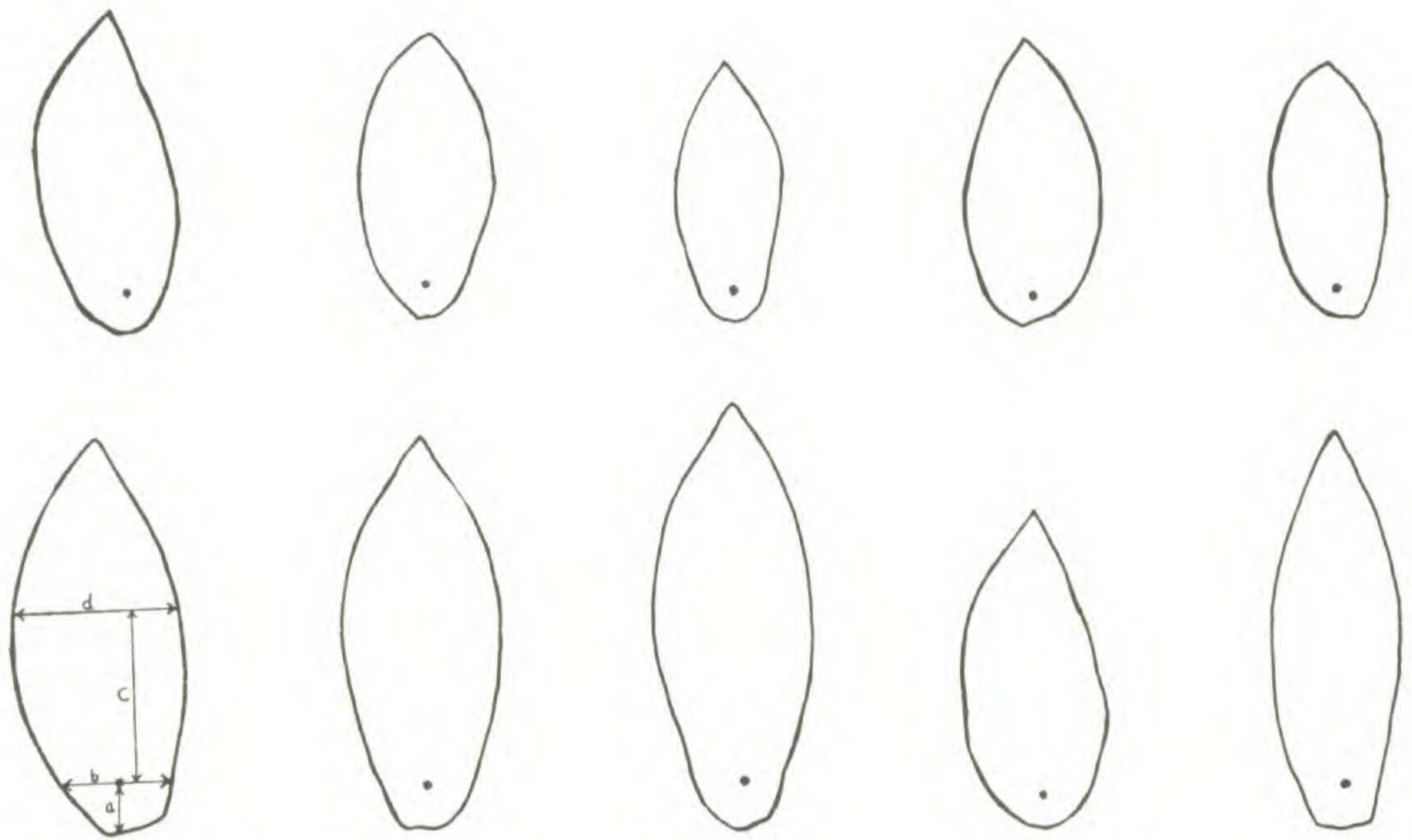


FIGURE 3. Leaf outlines from five specimens of *U. grandiflora* (above) and five of *U. perfoliata* (below). The four features measured are shown on the lower left hand specimen.

- A. The leaf of *U. grandiflora* is a little larger on the average.
- B. The leaf of *U. grandiflora* has a strong tendency towards something like the outline of a wooden shoe, with a narrow diameter through the heel and a broad one through the sole. This was made the subject of a more extended investigation, the results of which are reported in a later section.
- C. The leaf of *U. grandiflora* is usually pubescent on the under side. That of *U. perfoliata* is usually glabrous. Microphotographs of the leaf surfaces of 4 specimens of each species are shown in Plate 82. While this difference is a discontinuous one for the material considered in the present study, it would probably lose its discontinuity if a larger series of each species were examined. The specimen from Creve Coeur Lake, Missouri, was practically glabrous though it was in other respects a typical plant of *U. grandiflora*. Nor is there any evidence that it might have resulted from hybridization. The region in which it was collected

is far outside the natural range of *U. perfoliata* and the particular locality is a semi-public reservation in which the original vegetation has been practically undisturbed.

- D. *Uvularia grandiflora* has usually three veins in the leaf which are much larger than the rest. In *U. perfoliata* there are large veins and small ones, but there are seldom three which are clearly of another order of size than the remainder.

FLOWERS.

Photographs of representative flowers are shown in Plate 83. They exhibit a number of differences which are characteristic of the two species. If the flowers could be more easily examined without injury to the specimens, several of these characters would have been studied statistically. A survey of the 30 sheets shows the following differences.

- A. *Uvularia perfoliata* has large glandular outgrowths on the side of the perianth segments. These outgrowths are not present in *U. grandiflora*.
- B. In *U. grandiflora* the style is much longer in proportion to the stamens than in *U. perfoliata*.
- C. The perianth segments of *U. grandiflora* are longer on the average. The actual extremes were 2.4 — 4.7 cm. in *U. grandiflora* and 2.4 — 3.6 cm. in *U. perfoliata*. The respective medians for 7 specimens of each species are 3.2 and 2.7 cm.
- D. The stamens of both species have a pointed sterile tip. This tip is on the average much longer in *U. perfoliata*.

STATISTICAL ANALYSIS OF DIFFERENCES IN LEAF SHAPE.

Four measurements were taken on each leaf, the points to be measured having been chosen in such a way as to include the most outstanding differences in leaf shape between the two species. The four distances measured are indicated for the lower left-hand leaf of Figure 3. In interpreting this diagram it should be remembered that the leaf of *Uvularia* is perfoliate. The four measurements may be defined as follows:

- a. The distance from the stem to the rear margin of the leaf, along the median line.
- b. The width of the leaf at the stem.
- c. The distance from the stem to the point of maximum width.
- d. The maximum width at right angles with the mid vein.

The results are summarized in Table III. It will be seen that as far as absolute values are concerned, that there is very little difference

between the two species. Quite another picture is presented by the ratios between these four measurements. There are six possible ratios and they are summarized in Table III. It will be seen that though in every case the two species overlap, their averages are quite different.

The three ratios in which the two species differ most markedly are a/d , b/c , and b/d . It was desired to utilize all three and in some way combine them into a simple ratio or index which would measure simultaneously all three ratios. This was done quite simply by use of the following formula: leaf index =

$$\sqrt{\left(\frac{a}{d}\right)^2 + \left(\frac{b}{c}\right)^2 + \left(\frac{b}{d}\right)^2}.$$

Figure 4 shows graphically the significance of the leaf index in terms of analytical geometry. Analytically the index is a diagonal through a box, each dimension of which is determined by one of the three ratios. The ratio a/d determines the width of the box, the ratio b/c its height, and the ratio b/d its depth. Since *U. grandiflora* on the whole has

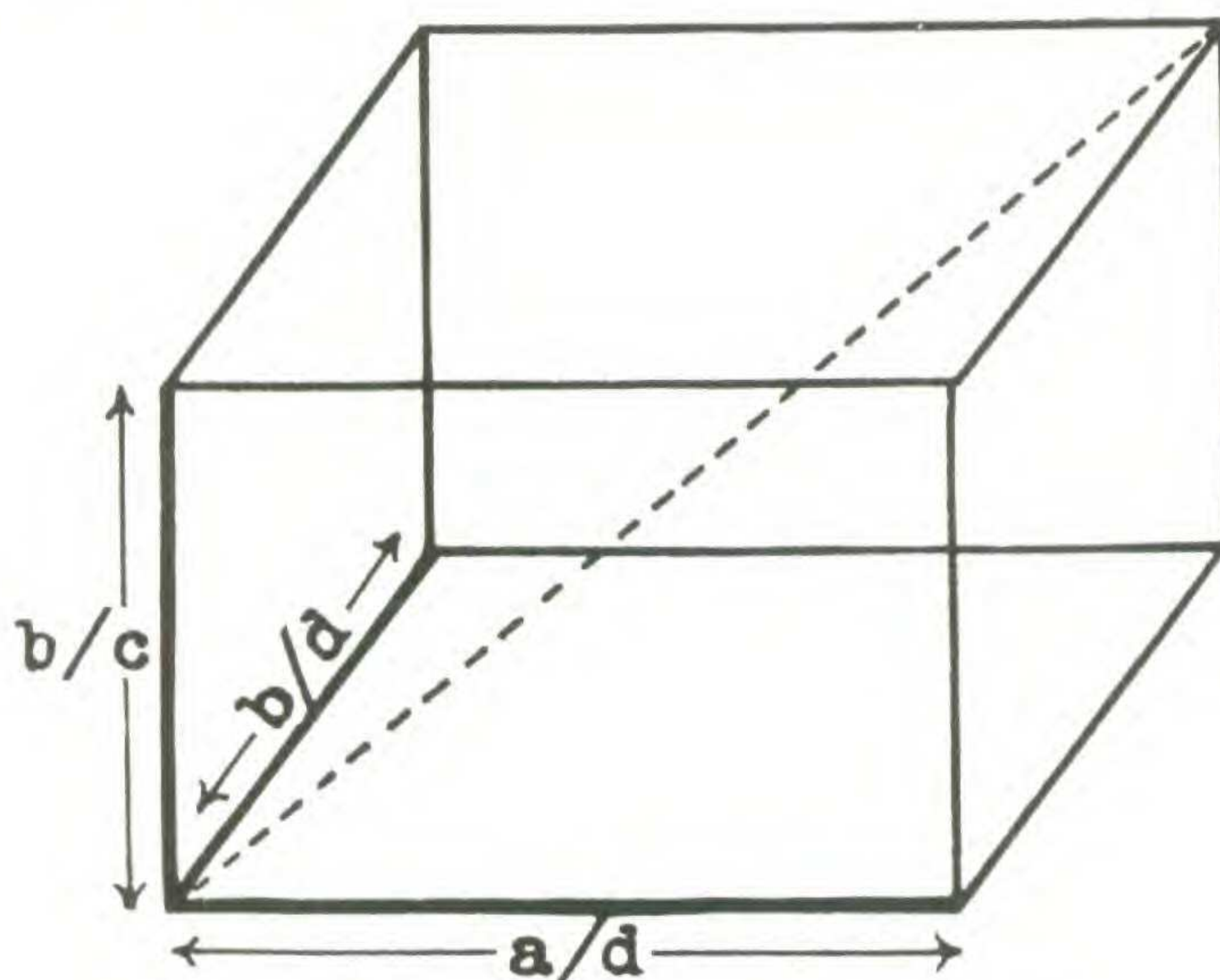


FIGURE 4. Explanation in the text.

lower values for all three ratios, the "boxes" are generally smaller, and the diagonals through these "boxes" generally shorter than is the case in *U. perfoliata*. The calculated values for the leaf index are collected in Table III. While they still give an overlapping distribution for the two species, they come much nearer to separating them than does any simple ratio taken by itself.

By the addition of two values calculated from characters of the stem, we can separate the specimens statistically. To accomplish this in a

simple way, we need characters for which the values of *U. perfoliata* are usually higher than those of *U. grandiflora* and which have a range of values roughly similar to those of the three leaf ratios (0.1 to 1.3). Characters fitting these requirements were obtained from, (1) the number of leaves below the lowest branch, and (2) the number of leaves on the sterile branch. In the case of the first, the actual number of leaves was divided by 10 and multiplied by 2, yielding values from 0.2 to 0.4 for *U. grandiflora* and from 0.4 to 0.8 for *U. perfoliata*. The new scale for the second character was obtained by subtracting the leaf number from 10 and dividing the result by 10. This yielded values of 0.2 to 0.6 for *U. grandiflora* and 0.6 to 0.9 for *U. perfoliata*. These two

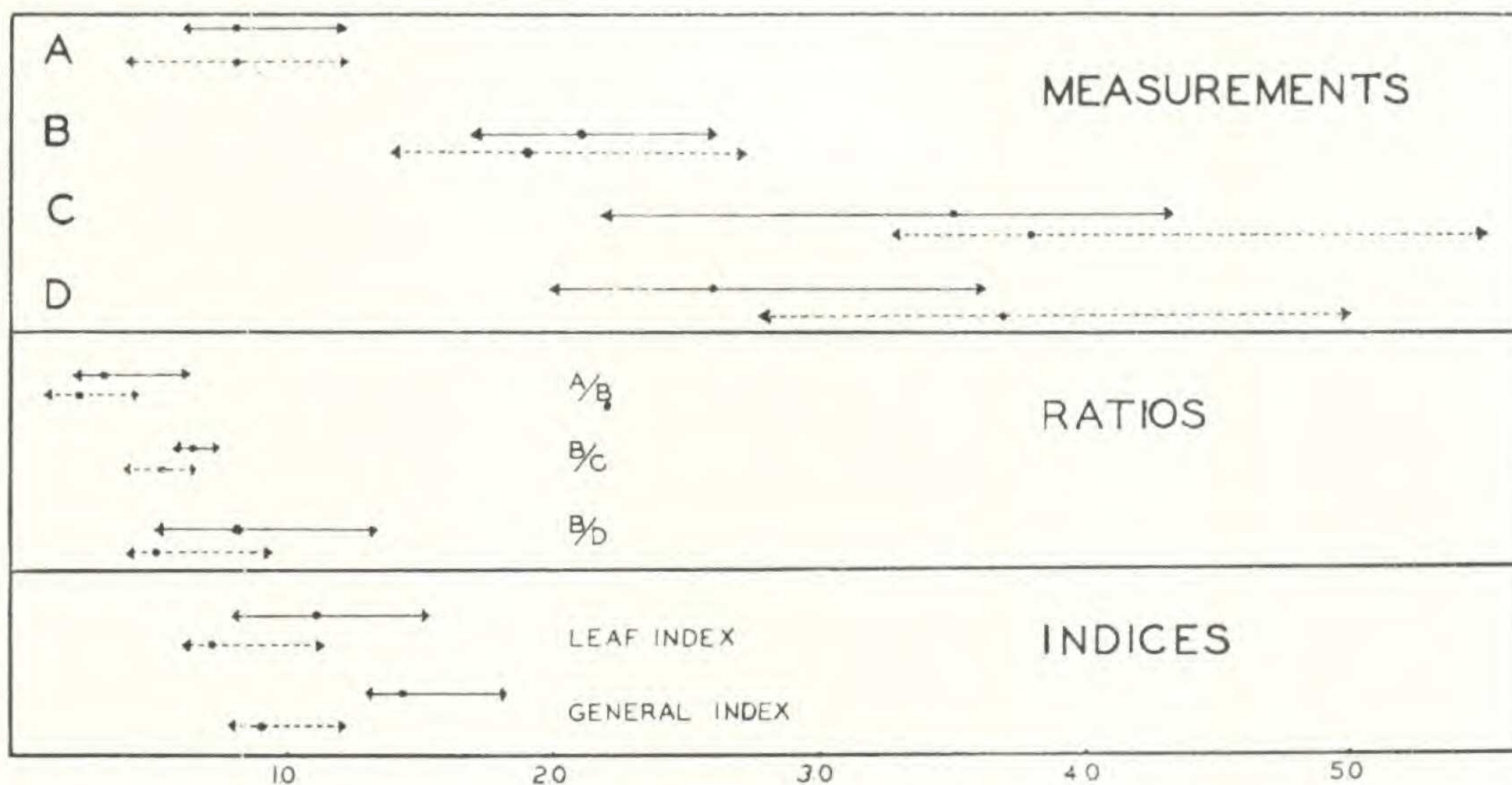


TABLE III. Variation in leaf measurements, ratios of measurements, and index numbers for 15 specimens each of *U. grandiflora* (dotted lines) and *U. perfoliata* (solid lines). The arrows show the limits of variation and the dots the position of the average values.

values may be added to the three ratios in the same simple way that was used in building up the leaf index, to form what we may call for lack of a better term the "General Index."

$$\text{General Index} = \sqrt{(a/d)^2 + (b/c)^2 + (b/d)^2 + (1)^2 + (2)^2}$$

Analytically we are still dealing with diagonals and although they are diagonals in multi-dimensional space, they are still capable of linear measurement, and the lengths of these diagonals may be grouped on a single scale as we have done in Table III.

It will be seen that the values of the "General Index" are completely discontinuous. In other words we have demonstrated statistically the

same point that was brought out in the graphical analysis of differences in the branching of the stem. *The species in question may be separated by a combination of tendencies even when none of these tendencies, taken by itself, will suffice.*

Quite as important as the morphological differences in a presentation



FIGURE 5. Distribution of *U. perfoliata* (open circles) and *U. grandiflora* (black dots).

of specific characteristics are physiological ones. For the most part they cannot be studied effectively with herbarium material. That they exist has been shown by Wiegand and Eames (1925) in their report on the two species in their "Flora of Cayuga Lake Basin." They state that *U. perfoliata* flowers two weeks or more later than *U. grandiflora*

and that it is found in "sandy acid or subcalcareous soil" while *U. grandiflora* grows in "gravelly or alluvial calcareous rich soils."

The ranges of species are in part, at least, determined by their physiological constitution. This point can, of course, be investigated with herbarium material and the distributions of the species of *Uvularia* as determined by that method are shown in Fig. 5. It will be seen that throughout the general calcareous areas of the Middle West, *Uvularia grandiflora* is a common species while in New England it penetrates only to the limestone areas of western Vermont. *U. perfoliata* conversely is a fairly common plant in New England and is found southward mainly along the mountains. There is an appreciable area where the two ranges overlap but for the most part they occupy different situations within that area.

DISCUSSION.

The above comparison of *Uvularia perfoliata* and *U. grandiflora* has shown four differences in the leaves, five in the nodes, and four in the flowers. Fundamental physiological differences were indicated by habitats and ranges, other morphological differences are known to exist in characters not available for study in our material. Of all the above differences, only three were discontinuous and there were indications that at least one of these would not have remained so if a larger series of specimens could have been examined. In the case of the intergrading differences, the two species could be separated by compounding several, no one of which would be sufficient if considered by itself.

If one attempts to think in general terms of the total hiatus between *U. grandiflora* and its nearest relative, he reaches a conception of specific differences as a combination of many minor tendencies. *The acknowledged discontinuity between the two species, taken in their entirety, is a discontinuity of combinations, reinforced by a few discontinuous differences in single characters.*

It does not seem to be generally realized that species may be, and customarily are, thought of in two quite different ways by different groups of biologists. Those biologists engaged in purely taxonomic work will unavoidably think of species in terms of the precise differences which permit their ready classification; which make it possible to arrange species in an herbarium or to construct a morphological key to a genus. To them the really essential differences between *U. grandiflora* and *U. perfoliata* will be those few discontinuous ones which are ordinarily used in identifying the species.

With this attitude of mind the present authors have no quarrel recognizing taxonomy as a difficult and necessary business, and that those who have it in hand are to be thanked for doing the work and must be allowed to develop their own methods of going about it.

On the other hand there are those who are interested not so much in the technical nature of the classifying process as in the biological make-up of the units which are being classified. This group will include some taxonomists, for the separation of the two lines of thought is not absolute.

To us, members of the latter group, the difference between two species is the difference between one kind of germ-plasm and another. As geneticists currently think of germ-plasms made up of an enormous number of separate units, so the difference between species, even between very closely related species, will be the resultant of a large number of minor differences. The seat of these differences is certainly the individual cells and will be expressed not in any one or two characters. It will extend throughout the plant, being more sharply manifest in some parts than in others.

From this point of view such tenuous characters as aspect and texture may have a very real biological basis. The characteristic leaf texture of a particular species, for instance, is ultimately dependent upon the size, form and arrangement, of the cells which make up the leaf. If the cells are small and regularly arranged, the leaf will have a close even surface; when the cells are irregular and large, the surface will be coarse and rough. Differences in aspect may in a similar way be traced back to the reactions of different germ-plasms.

Such specific differences, though slight and subtle, will on the whole manifest themselves similarly throughout the plant. Those internal forces which tend to produce small regular cells in the leaf will generally have a similar effect in the stem, and in the flower. The late Edward L. Greene has been credited with having separated species on the basis of "a certain indescribable grace." He may have been a good biologist, though a poor taxonomist, in relying on that distinction as an evidence of specific difference.

To us the many slight overlapping differences between species are more characteristic than the few sharply discontinuous ones. If we were forced to summarize the many differences between these two species of *Uvularia* in the fewest possible words, we could do no better than to say that *U. perfoliata* is neater and more delicate than *U. grandiflora*. In this way we might pack into a single phrase something

of the impression we have gained of two different life-stuffs, each of which reacts variously with the environment. Individual plants produced cooperatively by the germ-plasm and the environment, will show only one facet of the possibilities of that germ-plasm. For the full expression of the capabilities of a particular species there will be required a whole series of individuals produced under various environments.

SUMMARY

Part I.

1. Differentiation of species in the genus *Uvularia* has not involved gross differences in the number or relative sizes of the chromosomes.

Part II.

2. *Uvularia perfoliata* and *U. grandiflora* were chosen for a study of the nature of specific differences. A summary is presented of such differences as can be investigated in herbarium material.
3. A statistical method is described which compounds two or more separate measures into a single index. By the use of this method it is possible to separate the two species by a combination of characteristics, no one of which would suffice if considered separately.
4. The nature of specific differences is discussed in the light of these results.

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EXPLANATION OF THE PLATES

Plate 82

Lower leaf surfaces ($\times \pm 6$) of *Uvularia grandiflora* ('G') and *U. perfoliata* ('P'). The leaves in order from top to bottom are from the following 4 collections:

Lyon s.n. 6/12/1904

Palmer 17998

Palmer 19237

Greenman 2165

The leaves of *U. perfoliata* are from the following 4 collections:

Wright s.n. 6/7/1878

Williams s.n. 8/1/1899

Williams s.n. 5/30/1900

Meridith s.n. 5/27/1921

The reader's attention is called to the fact that this Plate is not so much a demonstration of points made in the paper as an attempt to present an objective summary of one kind of specific difference (namely leaf-texture). So far as possible the same lighting, magnification, and photographic development have been used throughout. The lack of sharpness in the cuts of *U. perfoliata* is not inferior photography; it is due to the fact that that species has a smooth leaf with a somewhat waxy surface. The photographs demonstrate a point which is in line with the main contention of the paper, viz., that the leaves are more easily distinguished by their general texture and appearance than by the single technical character (presence or absence of hairs) which is customarily used in keys and descriptions.

Plate 83

Enlarged photographs ($\times 6$) of *Uvularia* flowers from herbarium specimens. The stigmas have been retouched with India ink.

'P' = *U. perfoliata* (from Burnham s.n. 6/2/1907 Lake George, N. Y.)

'G' = *U. grandiflora* (from Williams s.n. 5/22/1908 Brandon, Vt.)

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